```
   1 TTTCCTCACTGACTATAAAAGAATAGAGAAGGAAGGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC

  91 ATGGCTATGATGGAGGTCCAGGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGTCTCTCTGT
   1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys

     GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTTTCTTAAAAGAA
     ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu

 271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
  61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys

 361 ATGATTTTGAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCCTAGTGAGAGAAAGAGGTCCNCAG
  91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln

 451 AGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAAGCAACACATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAA
 121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys

 541 ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGG
 151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly

 631 TTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATT
 181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle

 721 TACAAATACACAAGTTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT
 211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr

 811 TCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCAT
 241 SerIleTyrGlnGlyGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis

 901 GAAGCCAGTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGAT
 271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp

 991 GATACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAA
```

FIG. 1

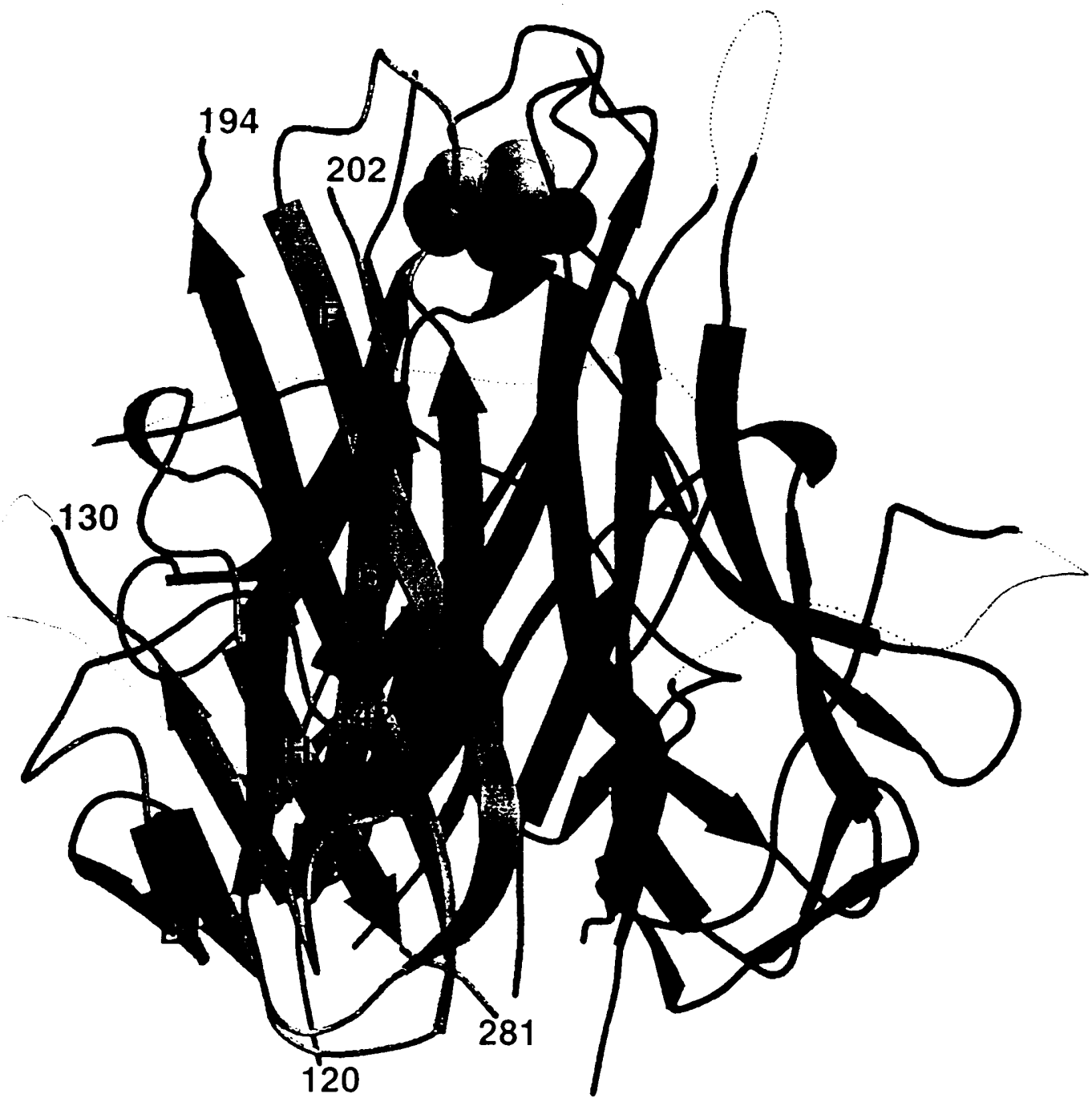



Figure 2A

Figure 2B

**Crystallographic Data**

| | Apc-2L(114-281) | Apo-2L(91-281)D218A | Apc-2L(91-281) D218A |
|---|---|---|---|
| Crystal | | | |
| Space Group | $P6_3$ | R32 | $R\bar{3}2$ |
| Unit Cell (Å) | a=72.5 c=140 | a=66.4 c=197.6 | a=66.4 c=197.7 |
| Resolution (Å) | 3.9 | 1.9 | 1.3 |
| Coverage (%) | 94 (96) | 93 (99) | 100 (100) |
| $\langle I/\sigma(I)\rangle$ | 5.9 | 10.1 | 12.4 |
| # unique (hkl) | 3589 | 12680 | 41840 |
| redundancy | 4.9 | 4.3 | 12.1 |
| $R_{symm}$ (%) | 15.4 (34) | 6.2 (27) | 6.4 (34) |
| # protomers in ASU | 2 | 1 | 1 |
| Refinement | | | |
| $R_{cryst}$ (%) | 33.8 | 20 | |
| $R_{free}$ (%) | 27.6 | 32 | |
| rmsd bonds (Å) | 0.009 | 0.015 | 0.007 |
| rmsd angles (°) | 1.79 | 2.0 | 1.41 |
| average B-values | -- | 14 | 14 |
| # water molecules | 0 | 170 | |

$R_{symm} = \Sigma_h\Sigma_i(I_{hi}-\langle I_h\rangle)/\Sigma_h I$ where $I_h$ is the mean structure factor intensity of i observations of symmetry-related reflections with Bragg index $h$. $R_{cryst}=(\Sigma_h\Sigma_i||F_{obs}|-|F_{calc}||)/\Sigma|F_{obs}|)$ where $F_{obs}$ and $F_{calc}$ are the observed and calculated structure factor amplitudes. $R_{free}= \Sigma_{(hkl)\in\tau}||Fobs_{(hkl)}|-k|F_{(hkl)}|/\Sigma_{(hkl)\in\tau}|Fobs_{(hkl)}|$ where the $\tau$ set of reflections is omitted from the refinement process. 10% of the data were included in the $\tau$ set for calculation of $R_{free}$ and not included in refinement. Values in parenthesis are for the highest resolution shell.

Fig. 2C


A
A′
B′
121
130
140
150
160
170
Apo2L
TNF-β
TNF-α
CD40L
FasL
RANKL
B
C
D
180
190
200
210
E
F
G
220
230
240
250
260
H
270
280

Figure 3

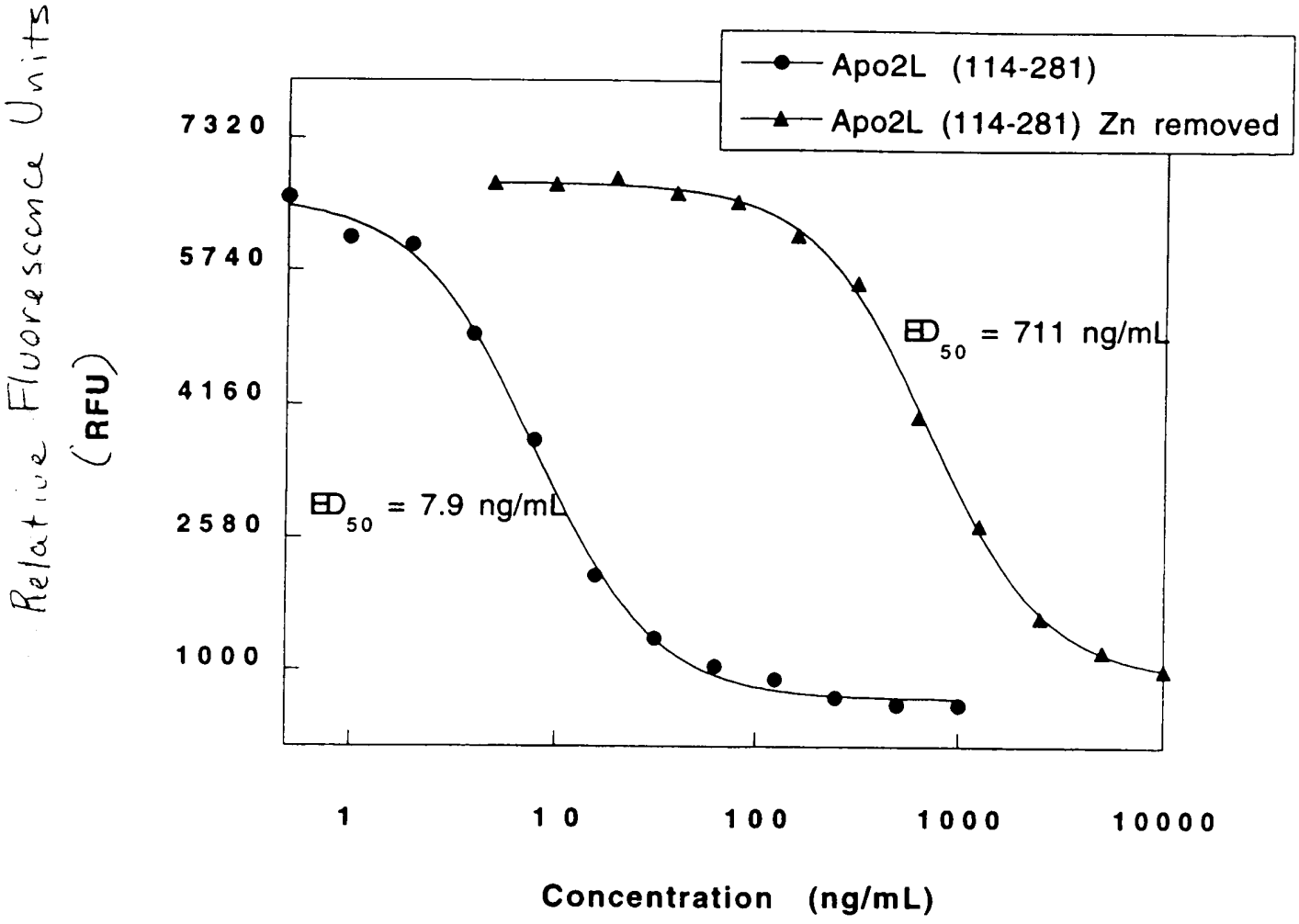

Relative Fluorescence Units (RFU)
7320
5740
4160
2580
1000
Apo2L (114-281)
Apo2L (114-281) Zn removed
ED50 = 711 ng/mL
ED50 = 7.9 ng/mL
1
10
100
1000
10000
Concentration (ng/mL)

Fig. 4

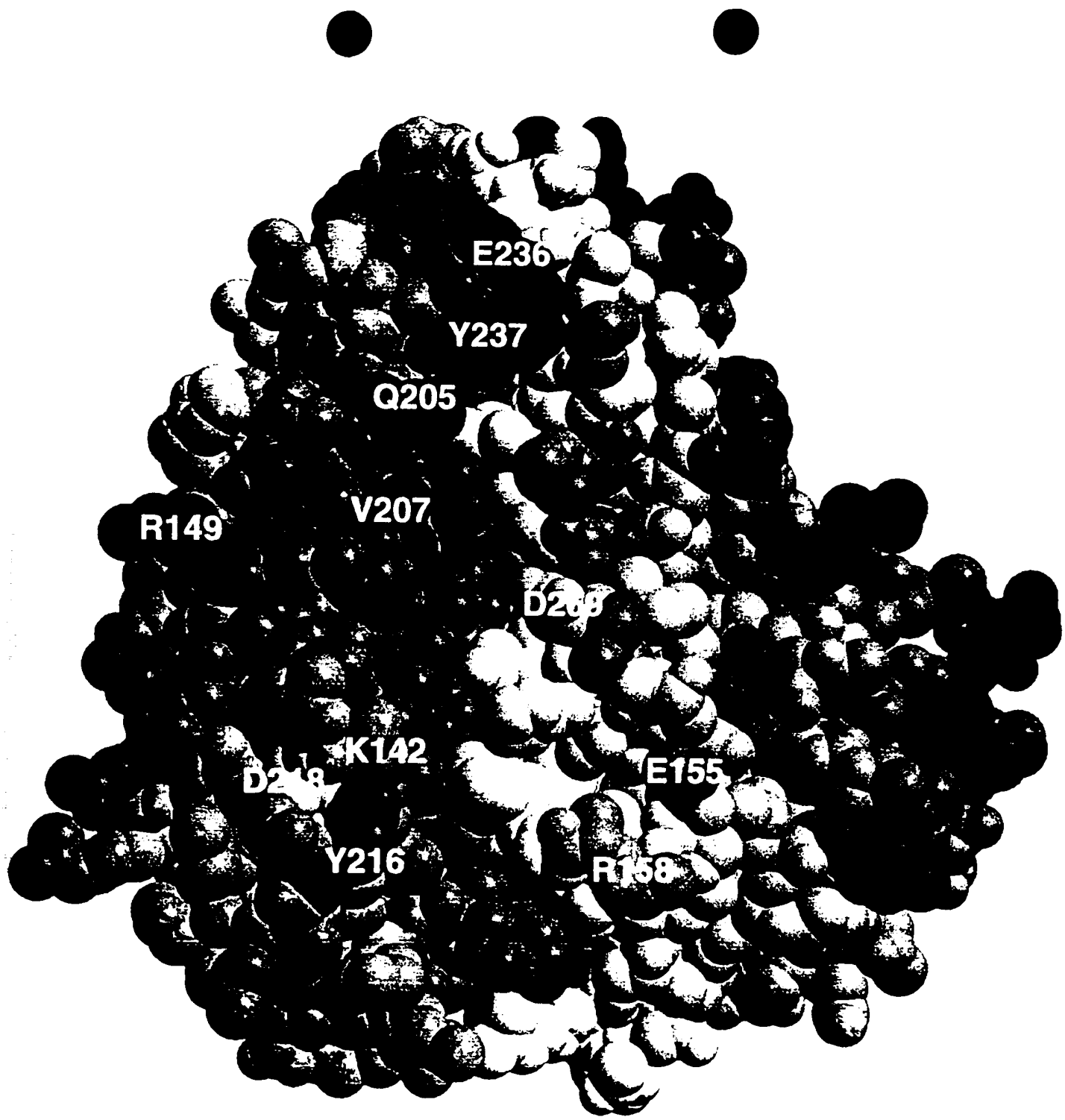



Figure 5

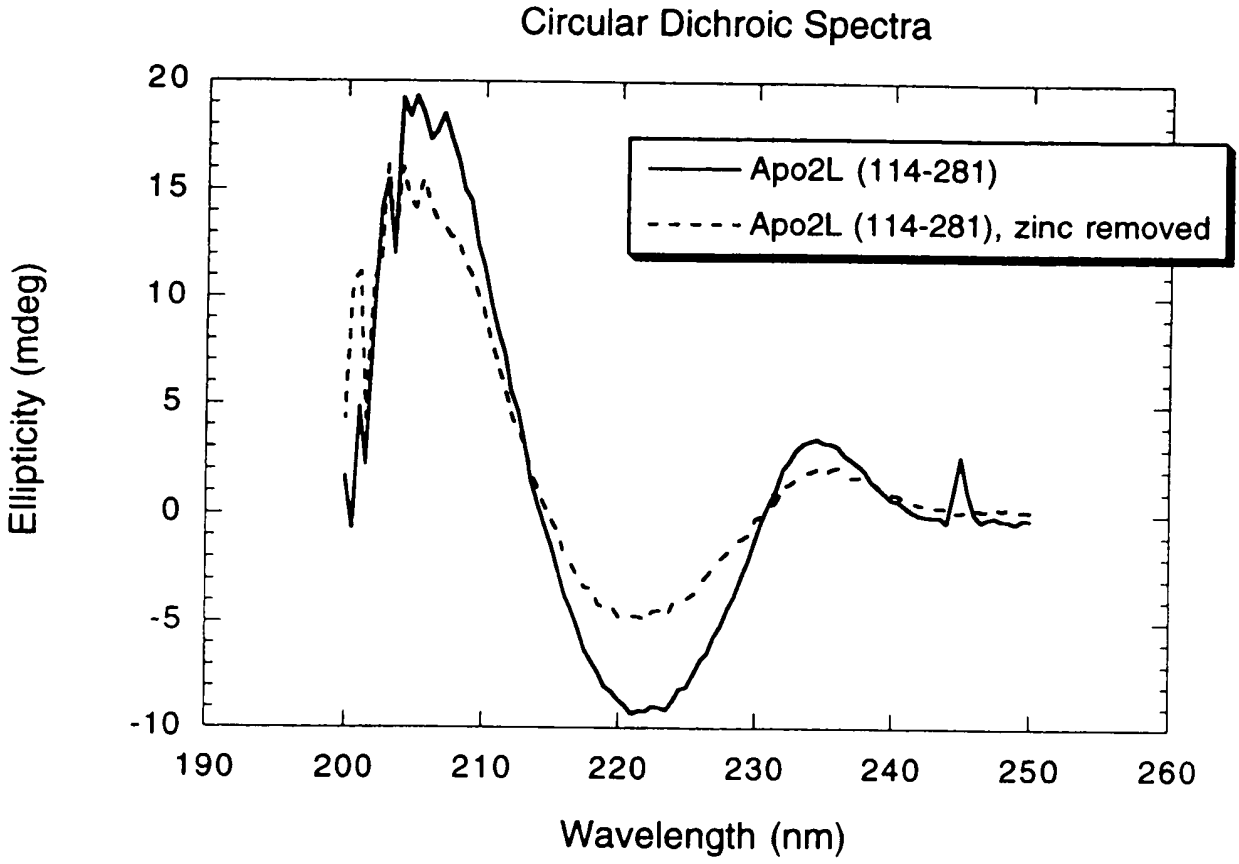

Circular Dichroic Spectra
Apo2L (114-281)
Apo2L (114-281), zinc removed
Ellipticity (mdeg)
20
15
10
5
0
-5
-10
190
200
210
220
230
240
250
260
Wavelength (nm)

Fig. 6

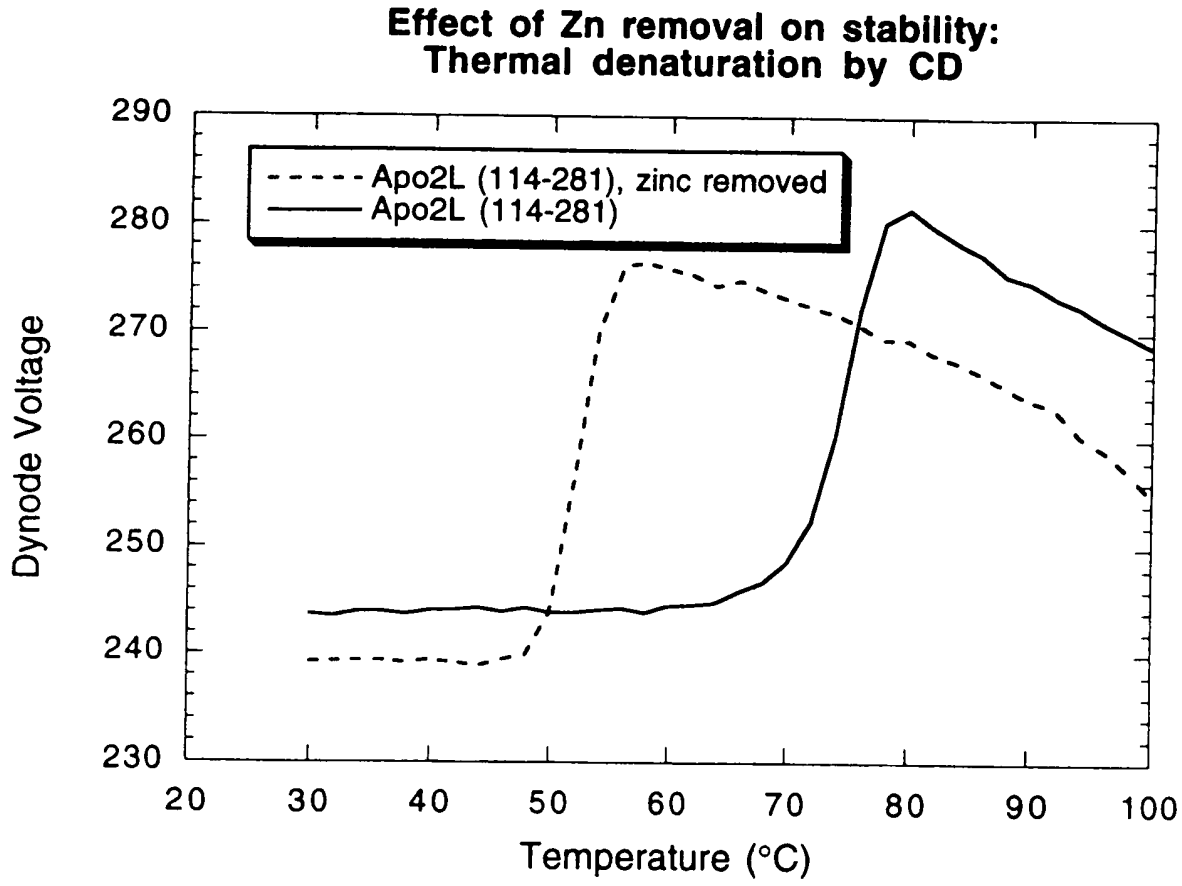

Effect of Zn removal on stability:
Thermal denaturation by CD
Apo2L (114-281), zinc removed
Apo2L (114-281)
Dynode Voltage
290
280
270
260
250
240
230
20
30
40
50
60
70
80
90
100
Temperature (°C)

Fig. 7

## Effect of ZnSO4 Additions on Apo2L Product Accumulation

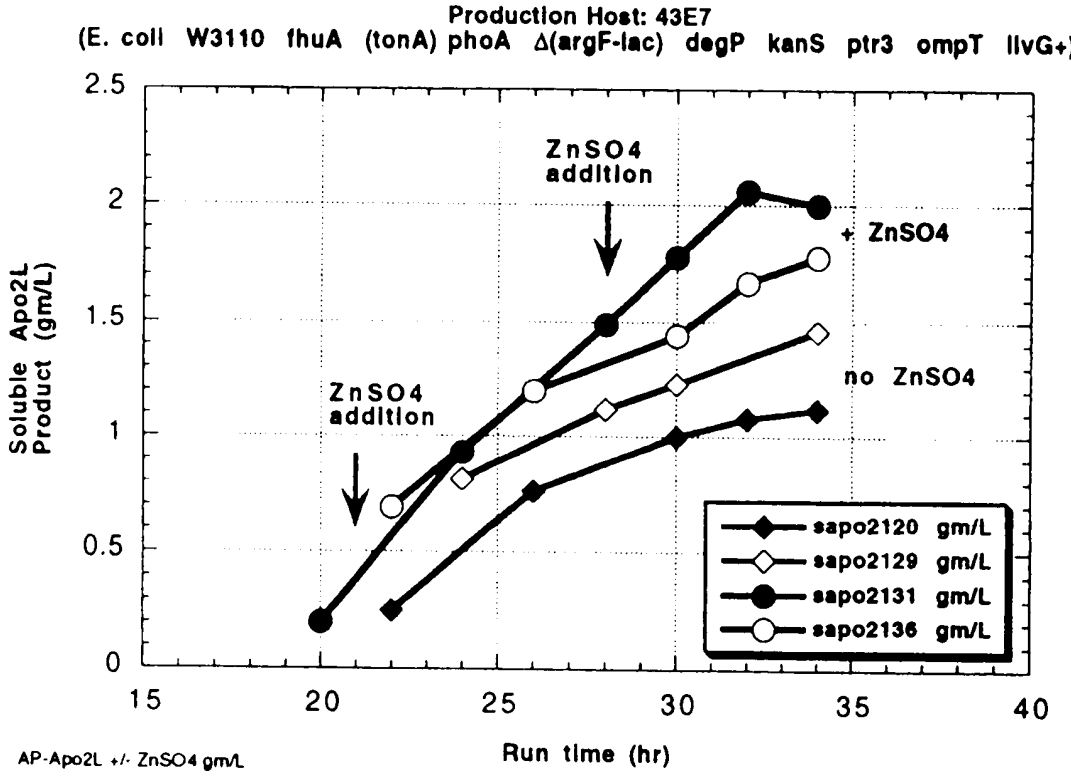
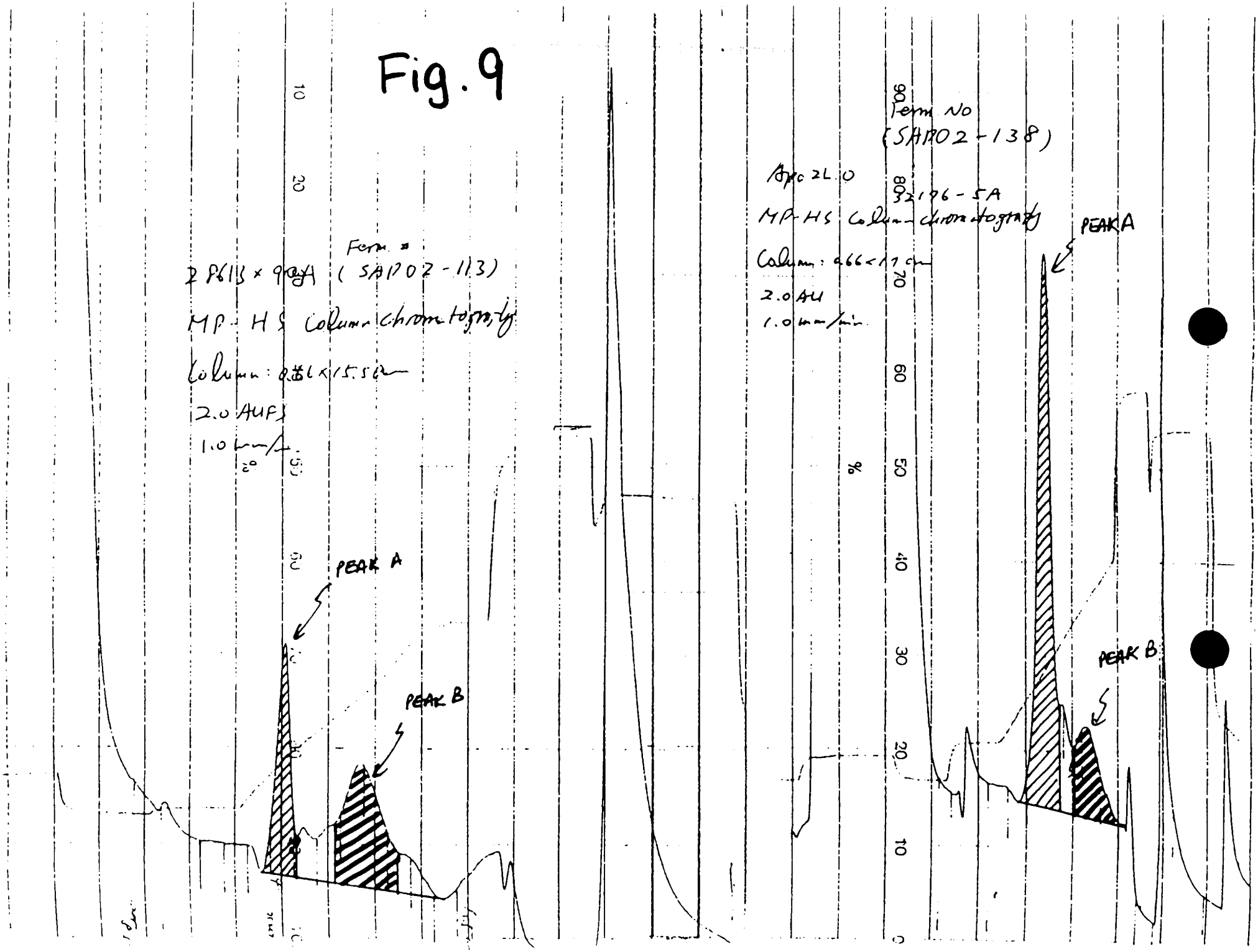



Fig. 8

# Fig. 9


Form No
(SA1102-113)
MP-HS Column chromatography
2.0 AUFS
1.0 mm/min
PEAK A
PEAK B
Form No
(SA1102-138)
MP-HS Column chromatography
2.0 AU
1.0 mm/min
PEAK A
PEAK B
%

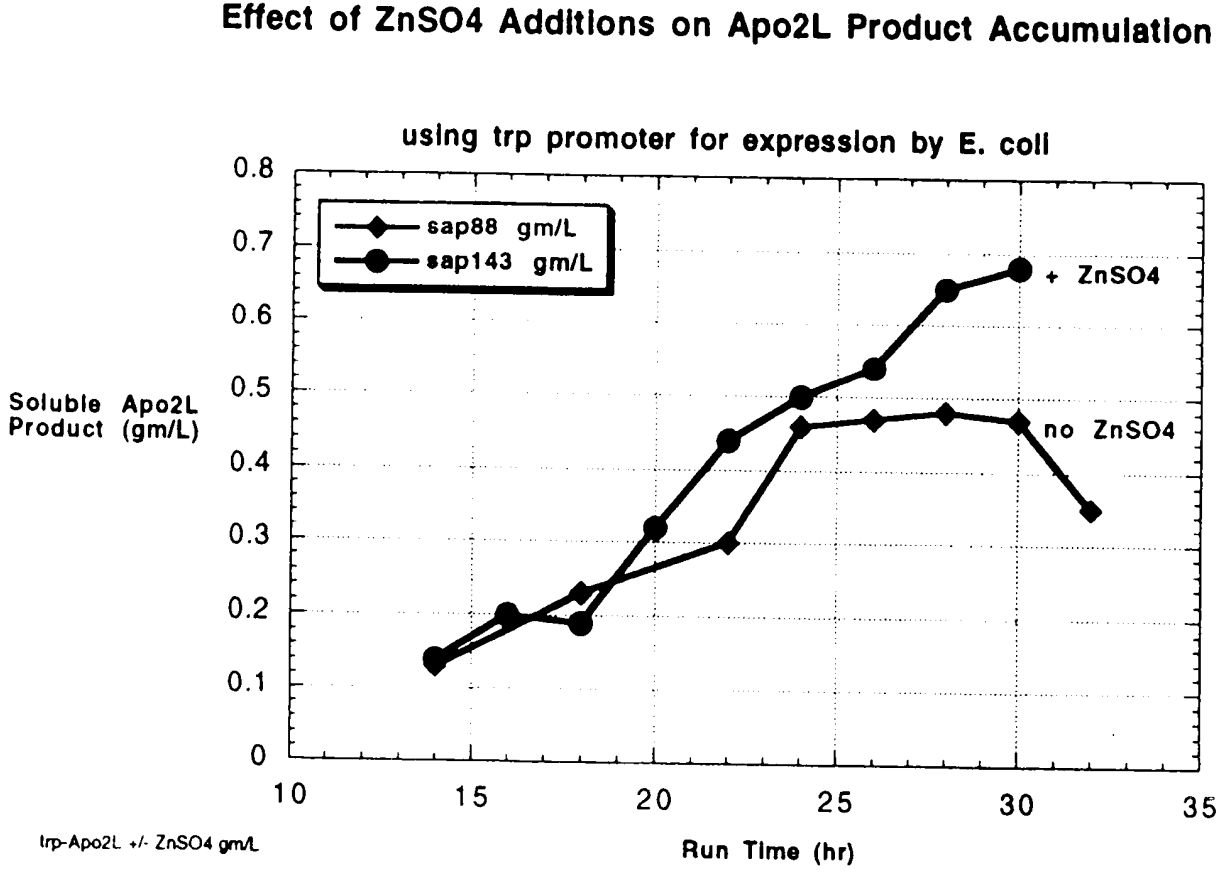

Effect of ZnSO4 Additions on Apo2L Product Accumulation
using trp promoter for expression by E. coli
Soluble Apo2L Product (gm/L)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
sap88 gm/L
sap143 gm/L
+ ZnSO4
no ZnSO4
10
15
20
25
30
35
Run Time (hr)
trp-Apo2L +/- ZnSO4 gm/L

Fig. 10

**Effect of CoCl2 additions on Apo2L Product Accumulation**

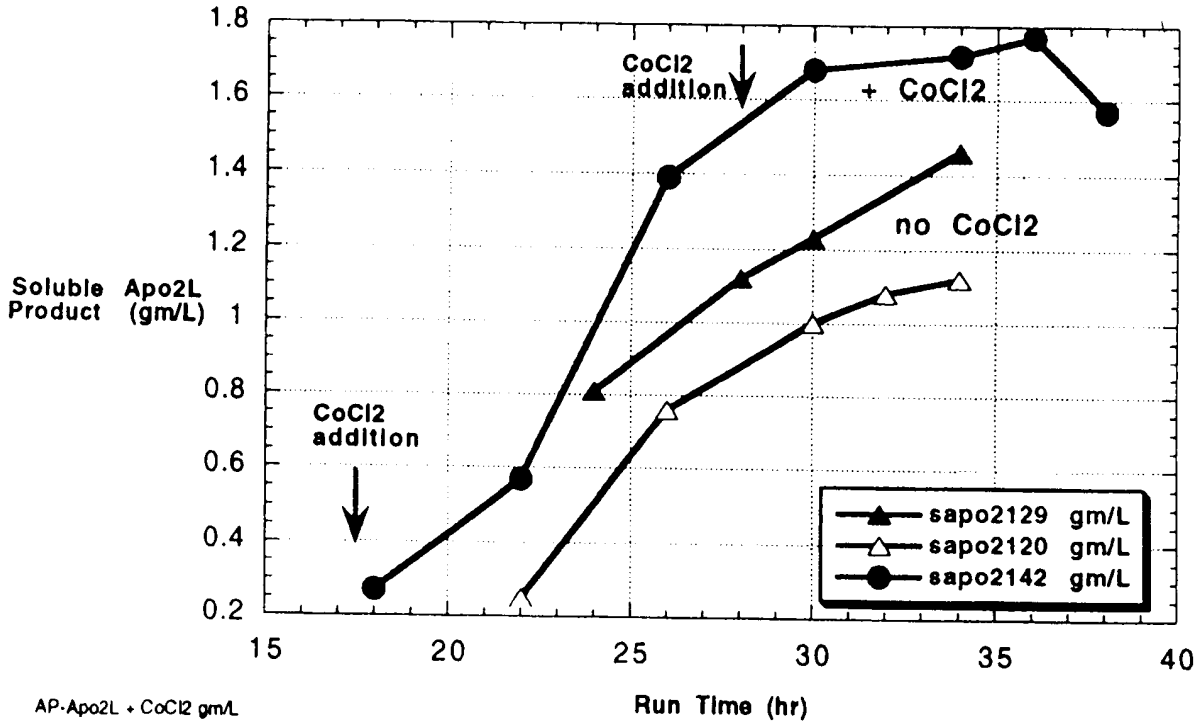



Fig. 11

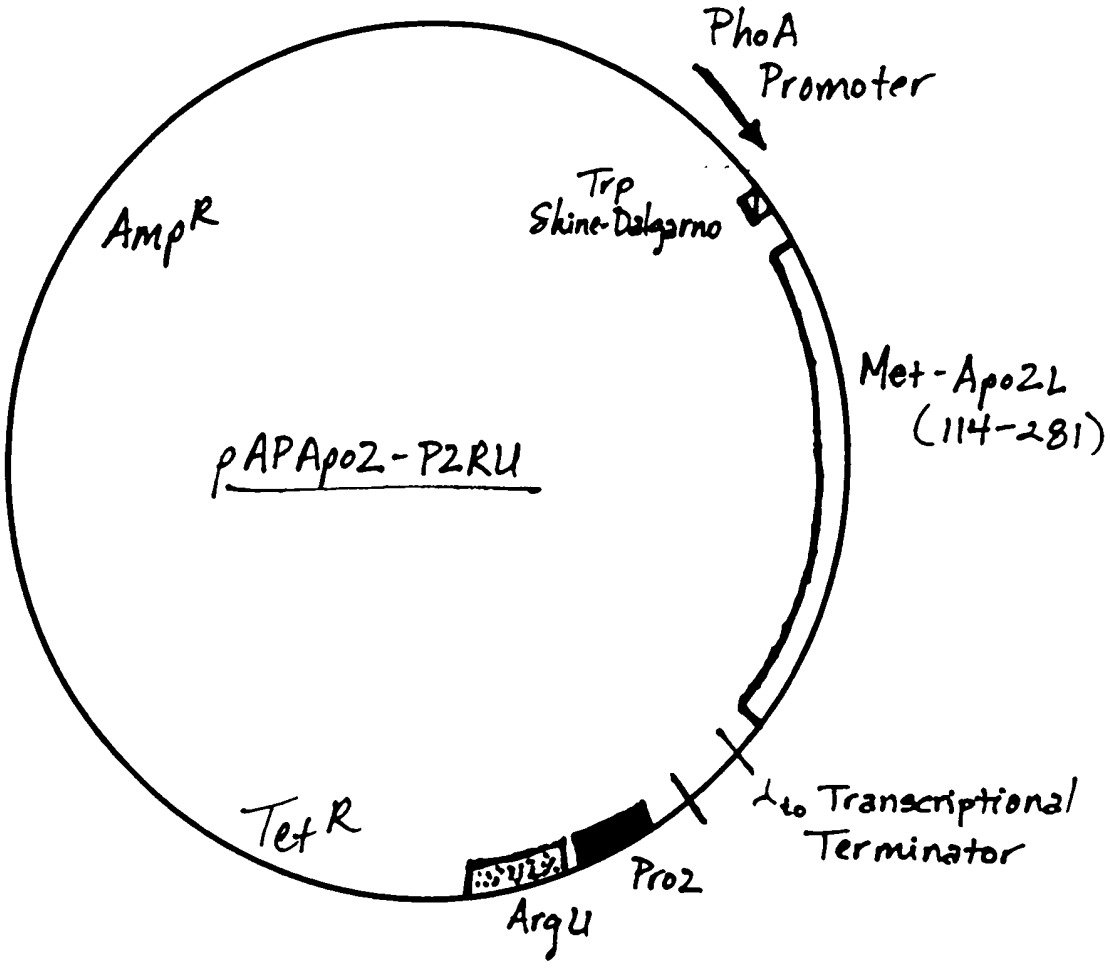

PhoA
Promoter
Trp
Shine-Dalgarno
AmpR
Met-Apo2L
(114-281)
pAPApo2-P2RU
λto Transcriptional
Terminator
TetR
Pro2
ArgU

Fig. 12

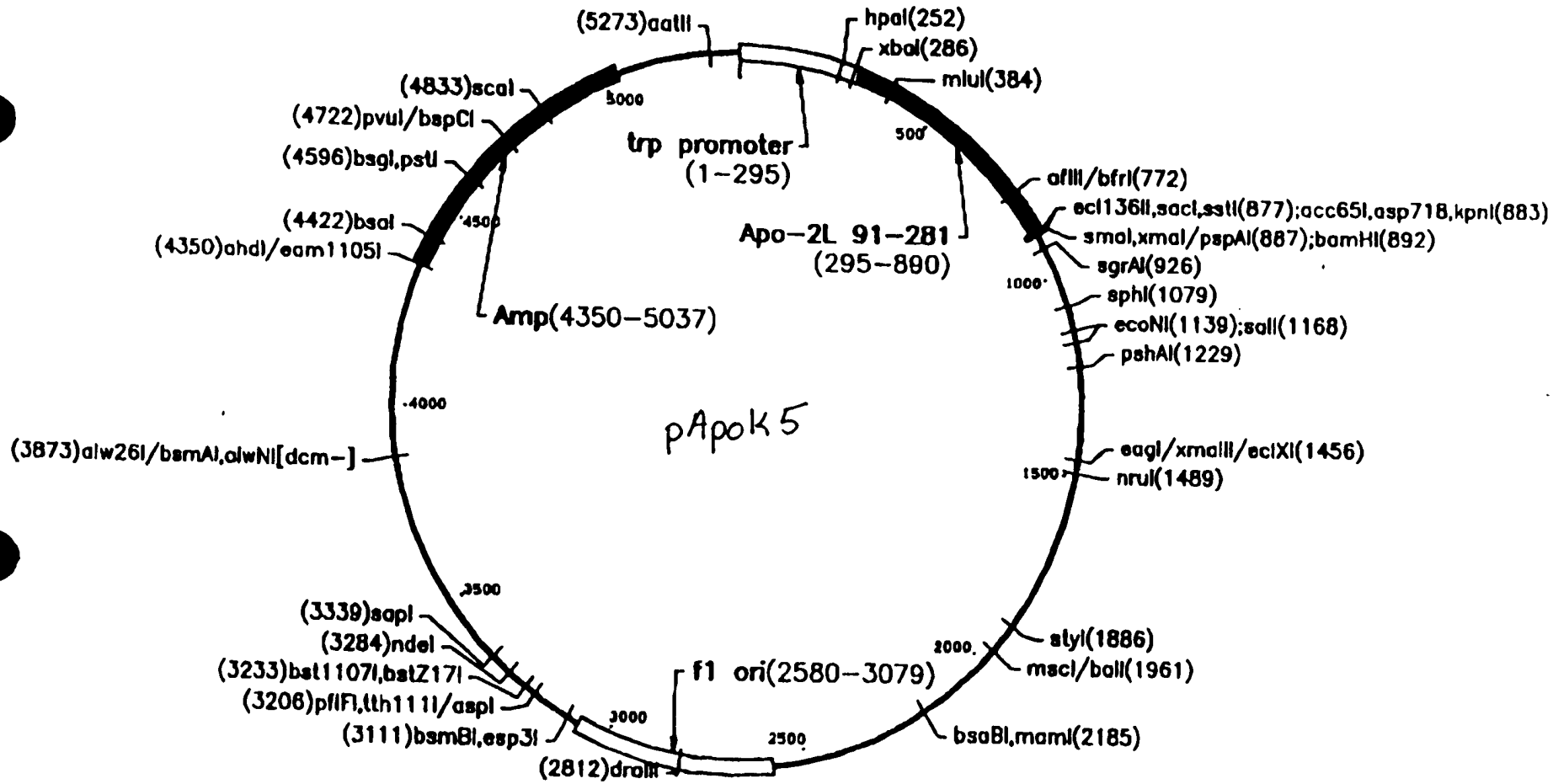

(5273)aatII
hpaI(252)
xbaI(286)
mluI(384)
(4833)scaI
(4722)pvuI/bspCI
(4596)bsgI,pstI
(4422)bsaI
(4350)ahdI/eam1105I
trp promoter
(1-295)
Apo-2L 91-281
(295-890)
Amp(4350-5037)
pApok5
aflII/bfrI(772)
ecl136II,sacI,sstI(877);acc65I,asp718,kpnI(883)
smaI,xmaI/pspAI(887);bamHI(892)
sgrAI(926)
sphI(1079)
ecoNI(1139);salI(1168)
pshAI(1229)
eagI/xmaIII/eclXI(1456)
nruI(1489)
styI(1886)
mscI/ball(1961)
bsaBI,mamI(2185)
f1 ori(2580-3079)
(2812)drdII
(3111)bsmBI,esp3I
(3206)pflFI,tth111I/aspI
(3233)bst1107I,bstZ17I
(3284)ndeI
(3339)sapI
(3873)alw26I/bsmAI,alwNI[dcm-]
500
1000
1500
2000
2500
3000
3500
4000
4500
5000

Fig. 13